DODAB/Monoolein Mixed Cationic Liposomes for Gene Delivery: Investigation by DLS, DSC, Fluorescence Spectroscopy and Phase Scanning Microscopy

I.M.S.C. Oliveira[1], J.P. Neves Silva[1], E. Feitosa[2], E.M.S. Castanheira[1], M.E.C.D. Real Oliveira[1]

[1]*Centre of Physics (CFUM), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.*
[2]*Physics Department/IBILCE, São Paulo State University, São José do Rio Preto - SP, Brazil.*

INTRODUCTION: Cationic vesicles are natural candidates for the delivery of genes into cells. The electrostatic attraction between positively charged lipid bilayers and negatively charged DNA enables the formation of lipoplexes through a compactation process that is driven by several physicochemical parameters [1]. The efficiency of DNA complexation is also enhanced by the inclusion of a co-surfactant (a *neutral lipid*) that functions mainly as a charge and geometry regulator for the liposomes.
In previous work, we have developed a novel liposomal formulation with high potential for gene delivery applications that contains the cationic lamellar forming surfactant dioctadecyldimethyl-ammonium bromide (DODAB) and the non-lamellar forming helper lipid 1-monooleoyl-rac-glycerol (monoolein, MO) [2]. In this study, we intended to gain further knowledge on the interactions between the lamellar and non-lamellar forming lipids used and how they affect the size and morphology of the aggregates, as a mean to better understand the DNA complexation and transfection efficiency.

METHODS: Liposomes were prepared by a modified ethanol injection method at a final concentration of 1 mM and different DODAB:MO molar ratios (4:1, 2:1, 1:1 and 1:2). Differential Scanning Calorimetry (DSC) was used to detect the melting transition of the lipid dispersions at different concentrations. The mean sizes (nm) of the mixed aggregates were measured by Dynamic Light Scattering (DLS). Structural changes were also detected by fluorescence experiments, through the excimer-to-monomer intensity ratio of the fluorescence probe Pyrene. Phase Scanning Microscopy (PSM) gave relevant information about the phase diagram of the mixed aggregates.

RESULTS: *Dynamic Light Scattering*: The mean size of the liposomes varies between 200-800 nm.

Differential Scanning Calorimetry: The DSC data show that, at 1.0 mM total lipid concentration, two kinds of aggregates are formed: one rich in DODAB (up to *ca* 0.2 mM in MO) that resembles the neat DODAB vesicles, with a melting temperature of T_m = 38 - 45 ºC, and another for higher MO concentrations (including neat 1.0 mM MO), exhibiting no transition peak.

Fluorescence experiments: The fluorescence probe pyrene gives indication of structural changes with increasing temperature that depend on the DODAB/MO molar ratio.

Phase Scanning Microscopy: PSM measurements (an example is shown in Figure 1) corroborate and complement the results obtained by the other techniques.

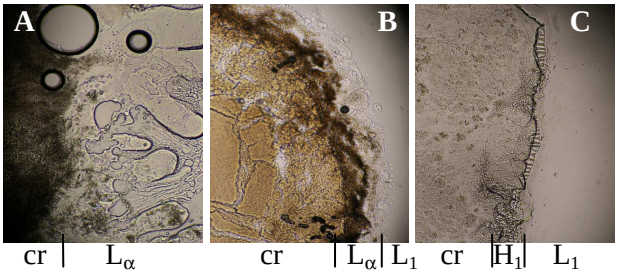

Fig. 1: PSM images for DODAB/MO mixed aggregates with different compositions. ***A****: 4:1,* ***B****: 2:1;* ***C****: 1:1. cr - crystalline;* L_α *- Lamellar;* L_1 *- cubic micellar;* H_1 *- hexagonal phases.*

DISCUSSION & CONCLUSIONS: The MO content has a strong influence on the structure and properties of DODAB/MO mixed cationic vesicles. This behaviour is mainly due to the ability of monoolein to form inverted non-lamellar structures, owing to its intrinsic negative curvature. This fact determines the efficiency of these mixed cationic vesicles to complex and further release DNA in gene delivery assays.

REFERENCES: [1]L. Xu, T.J. Anchordoquy (2008) *BBA* **1778**:2177–2181. [2]M.E.C.D Real Oliveira et al (2008) National Patent nº 104158; *Application of Monoolein as new lipid adjuvant for lipofection*, International Patent (PCT/IB2009/ 05361- PPI nr. 40759/09) submitted.

ACKNOWLEDGEMENTS: FCT and FEDER for financial support to CFUM and also by funding through project PTDC/QUI/69795/2006 and PhD grant of J.P.N. Silva (SFRH/BD/46968/2009).